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# Identification of Stem Rust Resistance in Three Synthetic Wheat Populations

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IDENTIFICATION OF STEM RUST RESISTANCE IN THREE SYNTHETIC WHEAT  
POPULATIONS

by

Russell Andrew Ward

A THESIS

Presented to the Faculty of  
The Graduate College at the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professor P.S. Baenziger

Lincoln, Nebraska

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# IDENTIFICATION OF STEM RUST RESISTANCE IN THREE SYNTHETIC WHEAT POPULATIONS

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University of Nebraska, 2012

Adviser: P. Stephen Baenziger

Wheat stem rust incited by *Puccinia graminis* Pers.:Pers. = *P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn is a major disease of wheat, with the potential to cause severe losses every year, hence making breeding for resistance important. For this project, two resistant synthetic wheat lines were studied using populations consisting of susceptible and resistant lines to determine the genetic basis of their stem rust resistance. Goodstreak has one dominant and one recessive resistance gene. Synthetics 303 and 370 each had two dominant genes present. For the Goodstreak/synthetic populations, testing  $F_2$  and  $F_{2:3}$  generations indicated the presence of three dominant genes and one recessive gene. To help identify individual genes, the synthetic lines were crossed to the cultivar 'Lorikeet' to test if the Ug99 resistance gene was *Sr33*, which is common in synthetic wheat lines, and the previously reported diagnostic marker Xcfd43 was used to test for the presence of *Sr6* hypothesized to be in Goodstreak. The resistant synthetic lines did not contain *Sr33*, but *Sr6* was in Goodstreak. We then postulated that the resistant lines contained one new *Sr33*-like Ug99 resistant gene and possibly *Sr9e* while Goodstreak contained *Sr6* and an unknown gene. By identifying, postulating, and observing four resistance genes, these sources of

resistance can be used and effectively incorporated in future cultivar improvement by wheat breeders to provide resistance to North American races of stem rust, as well as the Ug99 family of stem rust.

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## **FORWARD**

This thesis is written as one manuscript in the format required for publication in Crop Science journal.

## LIST OF ABBREVIATIONS

bp- Base Pair

CIMMYT - International Maize and Wheat Improvement Center

IT – Infection Type

GS274 – the cross Goodstreak/PI 648733

GS303 – the cross Goodstreak/PI 648758

GS370 – the cross Goodstreak/PI 648823

Syn274 – the synthetic line PI 648733

Syn303 – the synthetic line PI 648758

Syn370 – the synthetic line PI 648823



## TABLES LIST

Table 1. - Plant introduction number, CIMMYT identifier, and pedigree of six synthetic hexaploid wheats. Also listed are identifiers for *A. tauschii* donors at CIMMYT, KSU=Kansas State University, AUS= Australian Winter Cereals collection, and CPI= Commonwealth Plant Introduction collection.

Table 2. - Reaction based on Stakman et al. (1962) of six synthetic hexaploid wheats to two North American and four Ug99 stem rust isolates. Infection types of 2 indicate resistance, with 3 and 4 indicating susceptibility, + indicating more sporulation, and – indicating less sporulation (Onweller, 2011).

Table 3. - Infection type based on Stakman et al. (1962) rating scale of parental lines to stem rust isolates QFCS and TPMK, with ;, 1, and 2 indicating resistance, and 4 indicating susceptibility.

Table 4. - Segregation of *Sr6* alleles at the Xcfd43 microsatellite marker locus in three F<sub>2</sub> populations of GS274, GS303, and GS370.

Table 5. - Resistant and susceptible observed values, expected gene ratio for testing, the expected observation values and the resulting  $\chi^2$  value for F<sub>2</sub> individuals. Inoculated with stem rust race QFCS, unless noted in parenthesis.

Table 6. - Observed values, expected gene ratio, the expected values, and the resulting  $\chi^2$  value for grouped ITs in F<sub>2</sub> progeny of the population GS274 when tested with stem rust race QFCS.

Table 7. - Resistant and susceptible observed values, expected gene ratio, the expected observation values and the resulting  $\chi^2$  value for the F<sub>2:3</sub> families and F<sub>2</sub> plant ratios based upon the F<sub>2:3</sub> family ratios when inoculated with stem rust race QFCS.

## FIGURE LIST

Figure 1. - Migration pattern observed with the *Sr6* linked marker Xcfd43 in the parental lines of Goodstreak, Syn303, Syn274, and Syn370 with the following patterns scored (L to R): Lane 1- 100 bp ladder; Lane 2- Goodstreak containing *Sr6* band at 215 bp (indicated by arrow); Lane 3- Syn303 absent *Sr6*; Lane 4- Syn274 absent *Sr6*; Lane 5- Syn370 absent *Sr6*.

## LITERATURE REVIEW

Wheat (*Triticum* spp.) is one of the most important crops in the world in terms of nutrition and production. As the main ingredient of bread, cookies, cakes, and noodles, wheat provides more nutrients to the world than any other single food source, supplying approximately 19% of the world's caloric needs annually while being produced on nearly 217 million hectares worldwide (FAO, 2011; Pena, 2002). Of all the wheat grown, approximately 90 to 95% is planted as hexaploid bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ ) with the rest planted as tetraploid durum wheat (*T. durum* Desf.,  $2n = 4x = 28$ ) (Pena, 2002).

With the world's population projected to reach 9.3 billion people by the year 2050, it is paramount to produce more wheat while ensuring the safety of the world's wheat crop. One method to ensure the safety of the world's wheat crop is to protect it from diseases such as stem rust (*Puccinia graminis* Pers. F.sp. *tritici* Eriks. and E. Henn.) (United Nations, 2011).

Wheat stem rust, also called black rust, is a historically devastating disease of wheat that can result in widespread yield losses of 50-70%, with individual fields having up to a 100% loss (USDA-ARS Cereal Disease Laboratory, 2011). Stem rust has plagued wheat for thousands of years, as archeological excavations in Israel have discovered stem rust spores from the year 1300 B.C (Roelfs et al., 1992). Stem rust has also been described in ancient writings, as Aristotle (384-322 B.C.) described the "warm vapors" that produced rust, as well as the destruction of the crop by rust (Roelfs et al., 1992). Stem rust is also referenced in the Bible as one of the cereal rusts and smuts that affected the crops of the Israelites as punishment for their sins (Leonard and Szabo, 2005). Hence

from ancient times to the present, stem rust has been a problematic disease on wheat, causing epidemics in Africa, the Middle East, Australia, New Zealand, Europe, North and South America, and all of Asia except Central Asia (Saari and Prescott, 1985).

When speaking of plant diseases, the term epidemic is defined as a Change in disease intensity in a host population over time and space. The last major epidemic of wheat stem rust occurred in Ethiopia in 1993 and 1994, when a popular cultivar named “Enkoy” suffered severe yield losses (Singh et al., 2008). The last major epidemic of wheat stem rust to hit the United States occurred in 1986 in the central plains region. This epidemic was a result of the pathogen overwintering in a field along the gulf coast of Texas in combination with favorable moist, southerly winds to blow the pathogen north. Susceptible cultivars were widely grown in the central plains region, which resulted in yield losses of 5%-30% at harvest (Eversmeyer and Kramer, 2000). The worst epidemic of stem rust to affect the U.S. occurred in the North Central States in 1935. This epidemic resulted in yield losses of 56.5% in North Dakota, and 51.6% in Minnesota, both record losses (Roelfs, 1978; Leonard and Szabo, 2005). The two epidemics differed in terms of the stem rust lifecycle, as the epidemic of 1935 was caused by the pathogen overwintering on its alternate host barberry (*Berberis* spp.), in turn completing a full cycle and sexual recombination, leading to new virulent biotypes being formed. The latest epidemic of 1986 was the result of the pathogen overwintering on susceptible cultivars, thus infecting the crop from its asexual stage (Roelfs, 1978). Both epidemics were severe, but when the pathogen undergoes a full lifecycle with sexual recombination new races could be formed, thereby potentially causing more epidemics in the future. Here it is important to alter the stem rust lifecycle to prevent sexual recombination.

In order to alter the stem rust lifecycle, one must first understand all five stages of the lifecycle. The wheat stem rust fungus is a heteroecious obligate biotroph with a macrocyclic lifecycle featuring five distinct spore stages occurring during asexual reproduction on wheat or other Poaceae hosts, and during sexual reproduction on common barberry (*Berberis vulgaris* L.) or an alternate host Berberidaceae species (Singh et al., 2008; Leonard and Szabo, 2005). The full stem rust lifecycle begins with an infected plant, with elongated blister-like pustules (uredinia) full of loose brownish-red urediniospores found on the leaf sheaths, awns, glumes, stem tissue, and leaves. Pustules typically form on the lower side of the leaf, but may occasionally penetrate the upper surface of the leaf (Singh et al., 2008).

As the growing season progresses and the infected plant matures, the uredinia convert into telia and start producing teliospores as part of the sexual stage of the life cycle. Teliospores are black in color, and give forth the name black rust. Teliospores are firmly attached to the plant tissue and are commonly left in the field on the crop residue to serve as specialized survival structures to survive the winter (Leonard, 2005). During the dormant period the first steps in sexual recombination occur. Each teliospore contains two nuclei per cell, and each nucleus has one set of chromosomes. The nuclei contain a + mating type, and a – mating type which are paired together in each nucleus. Once dormant, the + and – mating types fuse together to create a single diploid nucleus, containing two sets of chromosomes. The chromosomes pair and the nucleus undergoes meiosis to form four haploid nuclei. Meiosis is then suspended during the winter, only to resume with favorable spring temperatures. The teliospore then begins to germinate, and

the four haploid nuclei migrate to one of four developing basidiospores. The four nuclei then divide to produce two haploid nuclei per basidiospore (Leonard, 2005).

Basidiospore germination coincides with bud break and new leaf growth in the alternate host species Berberidaceae (Leonard and Szabo, 2005). When the basidiospores reach maturity, they are forcibly ejected and carried by air currents to infect the alternate host. Young leaves of common barberry are infected the most, as barberry leaves become resistant as the plant matures. This occurs when the leaf surface develops thick cuticles as the plant ages, thereby not allowing the penetration peg of the basidiospore germ tube to penetrate the surface of the leaf. When the basidiospore penetrates the cuticle, pycnia are formed on the upper leaf surface. (Leonard and Szabo, 2005).

Within the pycnium, pycniospores containing a single haploid nucleus are produced in a sugary nectar to function as male gametes, and monokaryotic hyphae are produced to function as the female gamete. Each gamete is either a + or a – mating type to prevent self fertilization, as the + mating type can only fuse with the – mating type. When a pycniospore finds a receptive hypha fusion occurs, allowing for the pycniospore to migrate through the hypha to the base of the pycnium. Nuclear division with paired + and – mating type nuclei causes the cells to change to a dikaryotic state to form an aecium (Leonard, 2005). Fertilization is often aided by insects as the insects will visit multiple pycnia to feed on the sugary nectar produced, and in the process they will help spread pycniospores. This increases the chance of pycniospores finding a receptive hypha, and completing fertilization (Roelfs, 1985).

An aecium will develop on the underside of the barberry leaf directly underneath the pycnium, with single celled dikaryotic aeciospores rupturing the epidermis of the leaf.

Aeciospores can infect the Poaceae host, but not the Berberidaceae alternate host (Leonard and Szabo, 2005). The formation of aeciospores also represents the genetic recombination of the sexual phase of the stem rust lifecycle (Roelfs, 1985). Genetic recombination is a very important aspect of the stem rust life cycle, as mentioned previously it can lead to new virulent races being formed. Without recombination, the pathogen would have to rely on rare mutations to form new virulent races. When aeciospores are disseminated to a Poaceae host, the spore germinates to form a dense mass of hyphae below the leaf epidermis. From the mat of hyphae, single celled urediniospores are produced to form a uredinium, and the full life cycle is completed (Roelfs, 1978).

At this stage the urediniospores can continue to infect the Poaceae host, and can be disseminated long distances to infect other grassy hosts. However, at this stage free standing moisture is essential for urediniospores to infect. Without 6-8 hours of dew or moisture from rain, germination cannot take place (Singh et al., 2008). In the presence of free moisture, urediniospores are very successful at causing infections. The spores can germinate at temperatures as low as 2°C, and at temperatures as high as 30°C. Once an infection has taken place, spores will continue to be produced at temperatures as high as 40°C (Roelfs et al., 1992). Urediniospores are also very efficient at traveling long distances by air currents to infect other Poaceae hosts. Though most spores are deposited within the crop canopy and in close proximity to the infected plant, a significant number of spores can become airborne and reach heights of up to 3000 meters (Roelfs, 1985). These spores can be relatively long lived, as they can survive being away from host plants for a period of several weeks. Rain then removes the spores from the air,



depositing them on the surface of healthy plants, often many kilometers away from the original infected plant. (Roelfs et al., 1992). This allows for a constant source of inoculum to infect plants every year.

Currently in the U.S., infection by urediniospores is the method in which stem rust infects plants. Infected plants growing in southern climates spread their spores northwards in what is commonly referred to as the “Puccinia Pathway”. Volunteer plants growing in fields and ditches along the Gulf Coast of the United States remain infected throughout the winter, as temperatures seldom are low enough to kill the pathogen. These plants then serve as a source of inoculum to plants farther north during the wheat growing season.

Historically, urediniospores were not the only method of stem rust infection in wheat in the U.S. Prior to the early 20<sup>th</sup> century, infection by aeciospores from barberry was a common occurrence and epidemics of stem rust were a hazard of farming. Action to curtail stem rust was not taken until the stem rust epidemic of 1916, where over 200 million bushels (approximately 5.4 billion kg) of wheat were lost in the U.S., drastically impacting national food stocks (Roelfs, 1982). When the U.S. entered World War I in the spring of 1917, there was great concern whether the U.S. could feed the population at home and the troops abroad if another stem rust epidemic occurred with the same severity as the previous year. To ease those concerns, a decision was made to implement a national barberry eradication program, starting in 1918 (Peterson Jr, 2003; Roelfs, 1982).

Barberry eradication was no new topic in 1918, nor was it the first time barberry was targeted for removal in the United States. In 1660, a law was passed in Rouen, France outlawing barberry bushes near wheat fields, and similar laws were passed in

Connecticut in 1726, Massachusetts in 1754, and Rhode Island in 1766 (Roelfs, 1982).

All of these laws were ahead of their time, as it was not until 1865 when the German scientist Anton de Bary formulated the connection between barberry and stem rust (Peterson Jr, 2003).

Barberry eradication served three major purposes. First, it would slow the onset of stem rust by roughly ten days by not having spores directly available near the wheat fields. Urediniospores would have to travel from southern climates to cause infections. Second, the overall inoculum level would be reduced. There were vastly more aeciospores present from the leaves of barberry than urediniospores in the air above the wheat fields. Last, the number of pathogenic races would be reduced, stabilizing the current pathogenic races. (Roelfs, 1982). Without genetic recombination, new virulent races would only be formed by mutation.

North Dakota and South Dakota were the first states to pass a law against growing barberry in 1917, and were followed by Michigan, Minnesota, Nebraska, and Colorado in 1918, and Illinois, Indiana, Iowa, Montana, Ohio, Wisconsin, and Wyoming in 1919. These states combined their efforts with the federal barberry eradication program starting in 1918, and were later joined by Washington in 1923, Missouri, Pennsylvania, Virginia, and West Virginia in 1935, and Kansas in 1955. In addition to the eradication acts, a federal quarantine was enacted in 1919 to prevent interstate movement and planting of susceptible barberry plants. Different species and cultivars of *Berberis*, *Mahonia*, and *Mohoberberis* were tested for reaction to *P. graminis*, and those species and cultivars found to be susceptible were not allowed to be sold commercially and were destroyed when found on farms (Roelfs, 1982).

To eradicate barberry, programs first relied on farmers and youth services conducting farm to farm surveys for barberry plants, with detailed notes taken to illustrate where a plant was found. For a farm to be declared barberry free it required all barberry plants removed, and an annual inspection for new seedling growth for the following 15 years (Leonard, 2005).

In the 13 original states to outlaw barberry, the eradication focus was on areas directly bordering wheat fields as they had the greatest impact on infecting wheat. The effect of removing 100 large bushes from fence rows in wheat fields had a greater effect than removing 1,000 bushes from wooded areas (Roelfs, 1982). By 1933, over 18 million bushes had been destroyed in the eradication area (Leonard, 2005). In the following years, the frequency of rust epidemics began to decline, but the sexual cycle of stem rust was not yet broken. Although farmers removed a majority of barberry bushes around their fields, many were still present away from their fields. In 1953 and 1954 a new race of stem rust, race 15B, was able to overcome all deployed sources of resistance found in wheat. This race was found on a barberry bush near Fort Dodge, Iowa in 1939 (Leonard, 2005). This plant was far from any wheat field, proving the importance of removing all barberry plants, not just the plants surrounding a wheat field.

Once the focus was expanded to all areas, many years went by until the next stem rust epidemic occurred in 1974. This was a different kind of epidemic as it was caused by urediniospores overwintering in the Southeastern U.S. following a mild winter, and many susceptible cultivars were grown. By 1974, an estimated 100 million or more barberry plants had been eradicated. Starting in 1975, the federal barberry eradication program was gradually shut down (Roelfs, 1982). In 1981 the federal government turned

over full eradication responsibilities over to the states, and each state's eradication program was brought to a gradual stop, ending in 1990. The barberry quarantine is still in effect, and transporting barberry from state to state is still outlawed (Leonard, 2005).

Even though the threat of new virulent races coming from barberry has subsided, the wheat community cannot let its guard down on stem rust. In 1998, severe stem rust infections were observed in International Maize and Wheat Improvement Center (CIMMYT) nurseries at the Kalengyere Research Station in Uganda. When a race analysis was performed in the spring of 1999, it was discovered that the race had virulence on *Sr31*, indicating a new virulent race to which few wheat cultivars had resistance (Pretorius et al., 2000). This new race was named Ug99, or TTKS using the North American nomenclature system (Roelfs and Martens, 1988). The race was redesignated TTKSK when a variant of TTKS was found, and the nomenclature system added a fifth set of differentials to compensate. TTKSK was avirulent on *Sr24*, where a similar variant of TTKS was virulent on both *Sr24* and *Sr31*, was termed TTKST (Jin & Szabo, 2008). Today there are six different variants of the original TTKS race, with each having an almost identical DNA pattern, only differing in their avirulence/virulence formula (Singh et al., 2011).

As of 2011, Ug99 or a variant has spread to 8 additional countries, as far south as South Africa, and as far north as Iran (Singh et al., 2011). Predominant air currents in the Iranian region flow towards the east towards Pakistan and India, but also flow towards the north into the Caucasus and Central Asia. The eventual spread of Ug99 poses a serious threat to the world's production of wheat, as 25% of the world's wheat is grown

in this area, and an estimated 85%-95% of the worlds wheat germplasm is susceptible to Ug99 (Singh et al., 2006).

To protect against Ug99, breeders have made it a priority to incorporate new resistance genes into their germplasm. One unique method to accomplish this is the use of synthetic wheat. Synthetic wheat is made by intercrossing modern tetraploid durum wheat with derivatives of goat grass (*Aegilops tauschii* Coss.) to recreate the natural hybridization made thousands of years ago that resulted in modern hexaploid bread wheat (van Ginkel and Ogbonnaya, 2007).

The first events that led to modern hexaploid bread (AABBDD) wheat occurred approximately 500,000 years ago in the Fertile Crescent region of present day Iraq, Iran, and Turkey (Ogbonnaya et al., 2008; van Ginkel and Ogbonnaya, 2007). Around this time, a natural cross occurred between *A. speltoides* Tausch (BB) or a close relative (the actual B-genome donor is not known) and *T. urartu* Tumanian ex Gandilyan (AA) to form wild emmer (*T. dicoccoides* (Körn.) Körn. ex Schweinf, AABB). Once discovered, humans practiced selection for traits such as uniform crop establishment and growth, indehiscent seeds, and threshable seed. This domestication of wild emmer then resulted in cultivated emmer (*T. dicoccum* Schrank ex Schübl), which then evolved into modern durum wheat (van Ginkel and Ogbonnaya, 2007). About eight thousand years ago, it is estimated that natural outcrossing occurred between cultivated emmer (AABB) and goat grass (DD) to create modern hexaploid bread wheat (*T. aestivum*, AABBDD). This was a very important occurrence as this new crop contained gluten in its flour which when combined with yeast would rise, creating various forms of leaven bread. (van Ginkel and Ogbonnaya, 2007).

The natural cross between cultivated emmer and goat grass did not come without its drawbacks. Because of its low probability of occurring and with few individuals being formed, the cross created a genetic bottle-neck. The genetic diversity of wheat would continue to decrease as humans performed selection for favorable traits, only keeping desirable seed to plant the next season. The diversity of available disease resistance genes continued to erode as well, as the diseases evolved to become virulent on previously effective resistance genes (Ogbonnaya et al., 2008).

By resynthesizing modern bread wheat, novel genes are transferred into *T. aestivum*, creating new genetic diversity that was not present in the original cross or current *T. aestivum* gene pool. *Aegilops tauschii* is used because of its genetic proximity to the D genome of today's common bread wheat, in addition to being rich in genetic diversity of resistances to abiotic and biotic stresses (Assefa and Fehrman, 2004). To increase genetic diversity for diseases and other traits, breeders started to incorporate synthetic wheat into their germplasm. An early use of synthetic wheat in a breeding program was by CIMMYT to provide new resistance sources to karnal bunt (incited by *Tilletia indica* Mitra) (Dreisigacker et al., 2008). In addition to karnal bunt, synthetic wheat can provide new resistance genes to abiotic stresses such as drought, heat, pre-harvest sprouting, salinity, and waterlogging; pests such as the cereal cyst nematode (*Heterodera avenae* Woll.), root knot nematode (*Meloidogyne naasi* Franklin), Hessian fly (*Mayetiola destructor* (Say)), and greenbug (*Schizaphis graminum* (Rondani)); and to diseases such as leaf rust (incited by *P. triticina*), stripe rust (incited by *P. striiformis* Westend. f. sp. *tritici*), powdery mildew (incited by *Blumeria graminis* f. sp. *tritici* (DC.)

E.O. Speer f. sp. Em. Marchal), and especially stem rust (Schneider et al., 2008; van Ginkel and Ogbonnaya, 2007).

The D genome from *A. tauschii* is known to contribute three Ug99 resistant genes for stem rust resistance: *Sr33*, *Sr45*, and *Sr46* with additional, novel genes possible with more crosses between durum wheat and *A. tauschii*. With the emerging threat of stem rust to the world's wheat crop, it is important to have numerous, diverse sources of stem rust resistance. By incorporating new stem rust resistance genes from synthetic wheat, wheat breeders have a valuable resource to protect against stem rust, and to ensure the safety of the world's food supply.

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## INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world in terms of nutrition and production. As the main ingredient of bread, cookies, cakes, and noodles, wheat provides more nutrients to the world than any other single food source; supplying approximately 19% of the world's caloric needs annually while being produced on nearly 217 million hectares worldwide (FAO, 2011; Pena, 2002). With the world's population projected to reach 9.3 billion people by the year 2050, it is paramount to produce more wheat while ensuring the safety of the world's wheat crop. One method to ensure the safety of the world's wheat crop is to protect it from diseases such as stem rust (incited by *Puccinia graminis* Pers. f.sp. *tritici* Eriks. and E. Henn.) (United Nations, 2011).

Wheat stem rust is a historically devastating disease of wheat that can result in widespread yield losses of 50-70%, with individual field losses of up to a 100% (USDA-ARS Cereal Disease Laboratory, 2011). In recent years, stem rust losses have been minor in the U.S. due to the successful national barberry (*Berberis vulgaris* L.) eradication program and the widespread use of resistant cultivars. With the emergence of the virulent race Ug99 (race TTKSK using the North American nomenclature system) in Africa, wheat production in the U.S. and world is in danger once again due to this disease. (Jin and Szabo, 2008).

Ug99 has the ability to cause stem rust epidemics on a global scale, as an estimated 85%-95% of the world's wheat cultivars are susceptible to this race (Singh et al., 2006). To compound this risk, Ug99 has spread from Uganda into neighboring countries, and has mutated to form new races. As of 2011, Ug99 or a variant has spread

to as far south as South Africa and to as far north and east as Iran. Today there are six different variants of the original TTKSK race, with each having an almost identical DNA pattern, only differing in their avirulence/virulence formula (Singh et al., 2011).

To protect against Ug99, breeders have made it a priority to incorporate new resistance genes into their germplasm. One unique method to accomplish this is the use of genes from synthetic hexaploid wheat. Synthetic hexaploid wheat is made by intercrossing modern tetraploid durum wheat (*T. durum* Desf) with derivatives of goat grass (*Aegilops tauschii* Coss.) to recreate the natural hybridization that resulted in *T. aestivum* thousands of years ago (van Ginkel and Ogbonnaya, 2007). By recreating this cross, novel genes are transferred into *T. aestivum*, creating new genetic diversity that was not present in the original cross that created the current cultivated wheat gene pool. *Aegilops tauschii* is used because of its genetic proximity to the D genome of today's common bread wheat, in addition to being rich in genetic diversity of resistance/tolerance genes to abiotic and biotic stresses, particularly resistance to stem rust. (Assefa and Fehrman, 2004). *Aegilops tauschii* is known to have contributed three stem rust resistance genes: *Sr33*, *Sr45*, and *Sr46*. All three genes confer resistance to the Ug99 lineage of stem rust, though virulence to *Sr45*, and *Sr46* is known to exist in other stem rust races (Singh et al., 2011).

As part of a previous study, six synthetic lines were used in studies for drought tolerance. Pedigree information, as well as the *A. tauschii* parents of the synthetic lines used by other researchers with their identifiers are presented and cross listed in Table 1. In addition to being screened for drought tolerance, the six synthetic lines were screened for stem rust resistance at the USDA-ARS Cereal Disease Laboratory in St. Paul,

Minnesota. The synthetic lines were screened with the North American races TPMK and TTTT, and with the Ug99 family of TTKSK, TRTT, TTKST, and TTTSK, with the resulting infection types listed in Table 2 (Onwell, 2011). Two synthetic lines, PI 648758 (herein referred to as Syn303) and PI 648823 (herein referred to as Syn370), showed resistance to the Ug99 races and North American races of stem rust. At that screening, it was hypothesized both lines may carry *Sr33* due to it being common in synthetic wheats, and conveying resistance to Ug99.

The objective of this study was to determine the genetic basis of stem rust resistance in Syn303 and Syn370 as they may contain novel genes for resistance. To do so, testing populations were formed by crossing Syn303 and Syn370 to stem rust susceptible lines, and to a cultivar known to contain *Sr33*. In addition, as part of our drought research with the synthetic wheat lines, we had made crosses to ‘Goodstreak’ a line containing *Sr6* and an unknown gene (theorized to be *SrTmp*) (Baenziger et al., 2004; Jin and Singh, 2006). Goodstreak has been shown to be resistant to the North American races of stem rust, but not to Ug99. By identifying the stem rust resistance gene responsible for the Ug99 resistance, breeders can effectively deploy the gene to protect against the threat of Ug99.

## MATERIALS AND METHODS

The main plant materials were two drought tolerant and stem rust resistant synthetic lines (Syn303 and Syn370), a drought tolerant and stem rust susceptible synthetic line (Syn274), Bill Brown (a susceptible wheat cultivar), Goodstreak (a drought tolerant and stem rust resistant cultivar used in previous introgression studies with the drought tolerant synthetic wheat lines), and Lorikeet (a line containing *Sr33*).

To determine how many resistance genes were contributed by the parental lines, the synthetic lines of Syn303 and Syn370 were crossed to the cultivar ‘Bill Brown’, and Goodstreak was crossed to Syn274 (herein referred to as GS274). These were similar crosses, as Bill Brown and Syn274 both lack known stem rust resistance genes and are susceptible to all races of stem rust that we or others have tested, thus the resulting resistance genes in the F<sub>2</sub> populations will be inherited from the resistant parent (Haley et al., 2008; Onweller, 2011). For testing, 96 to 126 F<sub>2</sub> seeds of Syn303/Bill Brown and Syn370/Bill Brown, 216 F<sub>2</sub> seeds of GS274, the susceptible check ‘McNair 701’, and the parental lines of Syn274, Syn303, Syn370, and Goodstreak (also the resistant check) were planted for stem rust screening. The resulting seedlings were inoculated following the protocol described by Jin (2005) with stem rust race QFCS (avirulence/virulence formula *Sr6, 7b, 9b, 9e, 11, 24, 30, 31, 36, 38, Tmp, 1A.1R/ Sr5, 8a, 9a, 9d, 9g, 10, 17, 21, McN*) after the first and second leaves had fully expanded (Jin, 2009). Race QFCS was used as it is a less virulent race of stem rust, allowing for more resistance genes to be identified. Stem rust spores (race QFCS) were originally collected from a Nebraska field infection, then increased on McNair 701 and grown on stem rust differential lines to verify the race. After the race was verified the spores were collected and suspended in a

Tween 20 (40 ul per L of ddH<sub>2</sub>O) solution and dispensed from a pressurized spray bottle until the leaves were uniformly wet. The inoculated seedlings were then moved to a dew chamber with 100% humidity at 18°C for 18 hours, then transferred to a growth chamber set at 19°C for 16 hours of light and at 18°C for 8 hours of darkness. Because the cultivar Goodstreak possessed *Sr6*, and was used as a parent to produce the testing populations, it was necessary to keep the temperature below 20°C as *Sr6* is temperature sensitive and susceptible at high temperatures (Tsilo et al., 2009). Fourteen days after inoculation, the seedlings were removed from the growth chamber, and stem rust infection types (ITs) were scored using a 0-4 scale as described by Stakman (1962). Low ITs of 0, 1, and 2 were considered resistant, and high ITs of 3 and 4 were considered susceptible. Segregation ratios of resistant and susceptible plants were analyzed using the Chi-square test ( $\chi^2$ ) to determine the number of resistance genes present. After screening, the seedlings of GS274 were treated with a fungicide to control the stem rust infections, and then grown to maturity in a greenhouse.

Once mature, the F<sub>2</sub> plants of GS274 were harvested and a second stem rust screening with stem rust race QFCS was conducted on the F<sub>2:3</sub> families to confirm the F<sub>2</sub> ITs, and to distinguish the homozygous F<sub>2</sub> plants from the heterozygous F<sub>2</sub> plants. Sixteen to 32 plants per family were screened, with the susceptible check McNair 701 and the resistant check of Goodstreak included, using the same inoculation and rating procedure as the first rust screening. Segregation ratios of homozygous resistant, heterozygous, and homozygous susceptible families were analyzed using the Chi-square test ( $\chi^2$ ).

Additional testing was conducted with F<sub>2</sub> seed from the cross between Syn370 and Bill Brown using the North American stem rust race TPMK (avirulence/virulence formula *Sr6, 9a, 9b, 24, 30, 31, 38, 1A.1R./Sr5, 7b, 8a, 9a, 9d, 9e, 10, 11, 17, 21, 36, Tmp, McN*) to further understand the genetics of the resistance from the synthetic lines. F<sub>2</sub> seed from the cross between Syn303 and Bill Brown was not tested with race TPMK, as there was not enough seed available for testing. TPMK was used as it is more virulent than QFCS, which would test whether a gene present in the synthetic lines was resistant to stem rust race QFCS, but susceptible to race TPMK, to aide in identification of the genes present (Jin, 2009). TPMK was not used in earlier tests, as the priority was to identify the number of genes present using a less virulent race.

Once the number of genes from the parental lines was established, the focus was shifted towards better understanding the genetics of the resistance in the populations derived from crossing Goodstreak to the synthetic lines. To establish the F<sub>2</sub> populations, the two resistant synthetic lines of Syn303 and Syn370 (with the hypothesized gene *Sr33*), were crossed to the Nebraska cultivar Goodstreak, containing *Sr6* and an unknown gene (theorized to be *SrTmp*) (Baenziger et al., 2004; Jin and Singh, 2006). The resulting F<sub>1</sub> seed was then grown and selfed to generate the F<sub>2</sub> seed for this project. To determine the number of resistance genes present in each population, 106 F<sub>2</sub> seeds of Goodstreak/Syn303 (herein referred to as GS303) and Goodstreak/Syn370 (herein referred to as GS370) plus checks were planted, inoculated, rated, and analyzed using the same procedures as described in the first stem rust screening. After screening, the seedlings were treated with a fungicide to control the stem rust infections, and then grown to maturity in a greenhouse.



Once mature, the F<sub>2</sub> plants were harvested and a second stem rust screening with stem rust race QFCS was conducted on the F<sub>2:3</sub> families to confirm the F<sub>2</sub> ITs, and to distinguish the homozygous F<sub>2</sub> plants from the heterozygous F<sub>2</sub> plants. Sixteen to 32 plants per family plus checks were screened using the same inoculation and rating procedure as the first rust screening. Segregation ratios of homozygous resistant, heterozygous, and homozygous susceptible families were analyzed using the Chi-square test ( $\chi^2$ ).

To determine if the stem rust resistance genes in the synthetic lines were identical, allelism tests were conducted by crossing Syn303 to Syn370 to obtain F<sub>2</sub> seed. Two tests were completed, one using stem rust race QFCS with 230 F<sub>2</sub> seeds plus checks, the other with race TPMK using 100 F<sub>2</sub> seeds plus checks. Seedlings were inoculated, rated, and analyzed using the same procedures as described in the first stem rust screening.

An additional allelism test was used to verify if the gene present in the synthetic lines was *Sr33*, as hypothesized by the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota (Onwell, 2011). To do so, the synthetic lines of Syn303 and Syn370 were crossed to the Australian Cultivar ‘Lorikeet’ which contains *Sr33*. (Park and Bariana, 2008; CIMMYT, 2012). 850 F<sub>2</sub> seeds plus checks were planted, with the resulting seedlings inoculated with race TPMK, then rated and analyzed as previously described. TPMK was used in this test as the goal was to confirm whether the gene conveying resistance to Ug99 in the synthetic lines was *Sr33*, as both *Sr33* and the Ug99 resistant gene in the synthetic lines were resistant to TPMK. Additionally, TPMK was used to observe segregation in fewer plants in the event that *Sr33* was not the gene providing Ug99 resistance in the synthetic lines. Lorikeet contains resistance genes *Sr33*

and *Sr30*, both of which are resistant to TPMK and QFCS (CIMMYT, 2012), while the synthetic lines contained at least one gene resistant to both TPMK and QFCS, and one gene susceptible to TPMK while being resistant to QFCS. Thus if *Sr33* was not the Ug99 resistant gene found in the synthetic lines, a three gene segregation ratio would be observed in the F<sub>2</sub> population when tested with TPMK, needing at least 64 plants to observe one susceptible plant. A four gene segregation ratio would be observed if QFCS was used, needing at least 256 plants to observe one susceptible plant. By using TPMK, a smaller population size could be used to achieve the same results as using QFCS with a larger population size.

### **Molecular Analysis of *Sr6***

Leaf tissue was collected from young leaves from the parental lines of Goodstreak, Syn274, Syn303, Syn370; from the F<sub>2</sub> plants for GS303 and GS370; and from the F<sub>2:3</sub> plants for GS274. Genomic DNA extraction was conducted following the procedures described by Kuleung et al. (2004). The SSR marker Xcfd43 was previously identified as a diagnostic marker for *Sr6*, and the corresponding PCR protocol was followed as described in Tsilo et al. (2009). The SSR primer pair sequences were obtained from the GrainGenes website (GrainGenes, 2012 ). Polyacrylamide gel electrophoresis procedures were then followed according to Kuleung et al. (2004).

## RESULTS AND DISCUSSION

When tested with stem rust race QFCS, the stem rust resistant parent Goodstreak was highly resistant by displaying a fleck (;) IT, which is indicative of the resistance from *Sr6* (Table 3). The resistant parents Syn303 and Syn370 were moderately resistant, displaying an IT of 2. When testing the progeny, we will assume that any progeny displaying fleck “;” or 1 ITs will be from *Sr6* because ITs higher than ; have been identified in testing with *Sr6*. The *Sr6* IT can fluctuate based on the pathogen culture, temperature, and genetic background (McIntosh et al., 1995). In addition, consistently scoring a ; versus a 1 can be difficult. Infection types of 2 will be considered as non *Sr6* resistance genes. The stem rust susceptible parental lines Syn274 and Bill Brown were highly susceptible, both displaying an IT of 4.

To determine how many genes were present in the parental lines, Syn303 and Syn370 were crossed to Bill Brown, and Goodstreak to Syn274. These were similar crosses, as Bill Brown and Syn274 both lack known stem rust resistance genes and are susceptible to all races of stem rust that we or others have tested. Thus the resulting resistance genes in the F<sub>2</sub> populations will be inherited from the resistant parent (Haley et al., 2008 ; Onweller, 2011).

For the screening of the F<sub>2</sub> population of GS274, two genes were expected to be inherited from Goodstreak: *Sr6* and another hypothesized resistance gene, possibly *SrTmp*, with no resistance genes expected from Syn274 (Baenziger et al., 2004; Jin and Singh, 2006). To confirm the inheritance of gene *Sr6* from Goodstreak, the SSR marker Xcfd43 linked to *Sr6* was used. Migration patterns of the parental lines indicating resistance and susceptibility are displayed in Figure 1. In the F<sub>2</sub> population of GS274,

198 total plants were screened using the molecular marker, with 55 homozygous resistant, 99 segregating and 44 homozygous susceptible, fitting the expected 1:2:1 single gene segregation ratio ( $\chi^2=1.22$ ) and indicating the inheritance of *Sr6* marker from Goodstreak (Table 4).

To confirm the number of genes present in the GS274 population, 214  $F_2$  plants were tested with race QFCS, with 153 resistant and 61 susceptible plants observed (Table 5). For more insight into this segregation ratio, similar observed ITs were grouped together. Three types of ITs were observed: ;,1, 2, and 4. The ITs of ;,1 are indicative of *Sr6*, and the ITs of 2 are likely from the second gene in Goodstreak, which can convey a 2- to 2,3 infection type (McIntosh et al., 1995). When grouping the ITs into three groups, 117 ITs of ;,1 were observed, with 36 ITs of 2, and 61 ITs of 4, thus fitting a 9:3:4 ratio indicative of a dominant and recessive epistatic reaction involving two genes ( $\chi^2=1.57$ n.s.; Table 6). Three classes of infection types indicate that two genes are present, thereby supporting our original hypothesis.

To verify the initial ratings and to distinguish the homozygous plants from the heterozygous plants, additional stem rust screening was conducted in the  $F_{2:3}$  generation, with 209 families screened. One hundred fifteen were considered to be homozygous resistant, 57 segregating, and 37 homozygous susceptible (Table 7). This segregation fit a 9:4:3 ratio ( $\chi^2=0.61$ n.s) again indicating one dominant and one recessive gene, and that plants scored susceptible in the  $F_2$  generation were segregating in the  $F_{2:3}$  generation as the result of a recessive gene. To verify the initial ratings in the  $F_2$  generation, the homozygous resistant and segregating families were grouped together, resulting in 172

resistant families and 37 susceptible families. Again, the segregation ratios fit a one dominant and one recessive gene ratio of 13:3 ( $\chi^2=0.15$ n.s.).

Hence Goodstreak has two genes: *Sr6* and another gene. It had been speculated that the second gene might be *SrTmp*, however *SrTmp* is a dominant gene contrary to our segregation patterns. Validating that Goodstreak contains a recessive stem rust resistance gene can be determined by some plants scored susceptible in the F<sub>2</sub> generation to be observed as segregating in the F<sub>2:3</sub> generation. This result was observed in our data. From our testing in the F<sub>2:3</sub> generation, we were able to observe a more accurate number of susceptible families, thereby supporting our findings of one dominant gene (*Sr6*) and one recessive gene.

The results of our molecular marker and phenotypic data indicated that *Sr6* segregated in a 3 resistant (had the marker): 1 susceptible (did not have the marker) ratio. In addition, when the molecular marker data was compared against the ITs of the GS274 population, all of the *Sr6* markers were present in the resistant plants of the “9” class of the 9:4:3 ratio. The *Sr6* marker was absent in the susceptible plants of the 9:4:3 ratio, thus confirming that *Sr6* was the dominant gene.

To aide in determining how many resistance genes were contributed by Syn303 and Syn370, crosses were made between the synthetic lines and the cultivar Bill Brown. In the F<sub>2</sub> population of Syn303/Bill Brown, 77 plants were screened with race QFCS, resulting in 70 resistant plants and 7 susceptible, fitting a 15:1 two dominant gene ratio ( $\chi^2=1.06$ n.s.). Similar results were observed in the F<sub>2</sub> population of Syn370/Bill Brown, with 116 total plants screened, resulting in 110 resistant plants and 6 susceptible, also fitting a 15:1 two dominant gene ratio ( $\chi^2=0.23$ n.s.). With two resistance genes observed

in each synthetic line, the F<sub>2</sub> population of Syn370/BB was screened with the stem rust race TPMK to test if one or both genes were resistant to this race. 121 plants were rated with 95 resistant and 26 susceptible, fitting a single dominant gene ratio of 3:1 ( $\chi^2=0.80$ n.s.), thus indicating that one gene was resistant while the other was susceptible to race TPMK. No seed was tested with TPMK from the Syn303/Bill Brown cross, as there was not enough seed to test with, therefore we cannot postulate the number of TPMK resistant genes present in Syn303 from this test.

From our previous testing, we established that two genes were inherited from Goodstreak: one dominant gene (*Sr6*), and one recessive gene. We also determined that there were two genes inherited from the synthetic lines: one dominant gene resistant to QFCS, TPMK, and the Ug99 races of stem rust (possibly *Sr33* which is common in synthetic wheat lines), and one additional gene resistant to QFCS but susceptible to TPMK in Syn370 (Onweller, 2011). Therefore when tested with QFCS in the populations of GS303 and GS370, we postulated four total resistance genes to be present; three being dominant and one recessive in a segregation ratio of 253:3.

In the F<sub>2</sub> generation of GS303, 103 plants were tested with race QFCS, where 99 plants were observed to be resistant and 4 to be susceptible. This ratio did not fit our hypothesized segregation ratio of 253:3 ( $\chi^2=6.54$ ), but the population size was small. For the ease of scoring, plants rated 0-2 were considered resistant, while plants rated 3-4 were considered susceptible.

When the F<sub>2</sub> generation was tested with the diagnostic marker Xcfd43, 100 plants were analyzed resulting in 19 homozygous resistant, 55 segregating and 26 homozygous

susceptible, fitting the expected 1:2:1 single gene segregation ratio ( $\chi^2=1.98$ n.s.), confirming that *Sr6* was present within this population as well.

To gain more clarity on the number of genes present in this population, testing was conducted in the  $F_{2:3}$  generation. When the progeny were tested in the  $F_{2:3}$  generation, 99 families were screened with 76 considered homozygous resistant, 21 segregating, and 2 homozygous susceptible. When grouping the segregating and homozygous resistant families together, 97 resistant families were observed, with 2 susceptible. This supported our original hypothesis of having three dominant genes and one recessive gene ratio of 253:3 ( $\chi^2=0.62$ n.s.).

Similar results were observed in the  $F_2$  population of GS370 with 106 plants screened, resulting in 99 resistant plants and 7 susceptible plants observed. This also did not fit our hypothesized three dominant and one recessive gene ratio of 253:3 ( $\chi^2=27.00^{**}$ ).

When the  $F_2$  generation was tested with the diagnostic marker Xcfd43, 102 total plants were analyzed, resulting in 15 homozygous resistant, 52 segregating, and 35 homozygous susceptible. This did not fit the expected 1:2:1 single gene segregation ratio ( $\chi^2=7.88^*$ ), but did confirm the presence of *Sr6* in this population. The excess of susceptible bands could be explained by an anomaly in the inheritance of *Sr6* from Goodstreak when it was crossed to Syn370, as the marker Xcfd43 appears to be a useful marker, both from its published success in many other populations to verify *Sr6*, and from the observation of resistant and susceptible polymorphic bands between Goodstreak and the synthetic lines. It is possible that the anomaly could result from the preferential transmission of the Syn303 allele (*sr6*) at the *Sr6* locus.

When analyzed in the  $F_{2:3}$  generation of GS370, 101 families were tested with 85 considered homozygous resistant, 15 segregating, and 1 susceptible. When the homozygous resistant and segregating families were grouped together, 101 resistant families were observed, with 1 susceptible, fitting our originally hypothesized three dominant and one recessive gene ratio of 253:3 ( $\chi^2=0.03$ n.s.).

In both populations of GS303 and GS370, segregation ratios in the  $F_{2:3}$  families indicated that three dominant genes and one recessive gene were possible, thus fitting the expected inheritance of two dominant genes from the synthetics, and one dominant gene and one recessive gene from Goodstreak. As expected, both  $F_{2:3}$  populations fit additional segregation ratios, but the three dominant gene and one recessive gene was the only ratio fitting all of the data from the previous populations and parental lines. The recessive gene would cause plants scored susceptible in the  $F_2$  generation to be observed as segregating in the  $F_{2:3}$  generation. It was also observed that one plant in the population of Syn303 and two plants in the population of Syn370 were misclassified as susceptible in the  $F_2$  generation, whereas they were shown to be homozygous resistant in the  $F_{2:3}$  generation. It was also observed that one plant in the population of GS303 and four plants in the population of GS370 were classified as susceptible in the  $F_2$  generation, but were segregating in the  $F_{2:3}$ , again indicating a recessive gene.

The smaller population size likely could cause the additional segregation ratios to fit the populations, but testing with a larger population size would be needed to verify this suggestion. The small population size could also be a factor causing the differences between the results from the  $F_2$  and  $F_{2:3}$  generation in both populations. When the two dominant genes from the synthetic lines were combined with the one dominant and one



recessive gene from Goodstreak, it would be difficult to detect the recessive resistance gene in the  $F_2$  generation with the complex inheritance of the other three genes. Thus the combination of misclassified plants in combination with a small sample size and the affect of a recessive gene could cause a higher number of susceptible plants to be identified in the  $F_2$  generation. Testing in the  $F_{2:3}$  generation then enabled us to have a more accurate number of susceptible families, thereby supporting our findings of three dominant genes and one recessive gene.

When the  $F_{2:3}$  families were compared to the molecular marker data in both populations, all susceptible  $F_{2:3}$  families were confirmed as susceptible for *Sr6* by the molecular marker data. In addition, families determined to have the homozygous *Sr6* allele from the molecular marker data also had a ; infection type, indicative of *Sr6*. This supported the findings of our  $F_{2:3}$  generation, and confirmed the usefulness of the marker Xcfd43.

The next test was conducted to see if the genes were identical in the synthetic lines. To do so, Syn303 was crossed to Syn370. Two screenings were conducted with the  $F_2$  seed; one with race QFCS, the other with TPMK. In the screening with QFCS, there was no segregation observed within the 228 total plants, thus indicating that most likely one gene was identical in the synthetic lines. Race TPMK was used in the second screening of Syn303/Syn370, as both genes were shown to be resistant to QFCS from our testing in the populations of Syn303/Bill Brown and Syn370/Bill Brown, but only one was shown to be resistant to TPMK from our testing in the population of Syn370/Bill Brown. Because the population of Syn303/Bill Brown was not tested with TPMK, we do

not know the total number of TPMK resistant genes present in Syn303, but we know that there is at least one TPMK resistant gene present in Syn303 (Onweller, 2011).

Out of 96 total plants tested with TPMK in the population of Syn303/Syn370, no segregation was observed, again indicating the possibility that one gene in the synthetic lines was identical. Because there is only one gene in Syn370 that is resistant to TPMK, and earlier testing indicated that the Ug99 resistance gene was also resistant to TPMK, we can conclude that the Ug99 resistant gene was identical in each synthetic line. However, these tests could not verify if the TPMK susceptible gene was present in both synthetic lines, due to the presence of the other resistance genes.

Once the number of resistance genes in each population was determined, the focus shifted towards identifying the individual genes. To do so, the last stem rust screening was conducted to test if the synthetic lines' resistance to Ug99 was from *Sr33*. Both synthetic lines were crossed to the Australian cultivar 'Lorikeet', which contains one dominant gene (*Sr33*) and one partially recessive gene (*Sr30*) (CIMMYT, 2012; Jin, 2009). *Sr30* is reported to be partially resistant, so it is possible to see a range of values indicating that *Sr30* is either a dominant gene, or a recessive gene (Knott and McIntosh, 1978). Stem rust screening was conducted with race TPMK to reduce the number of F<sub>2</sub> plants needed for testing, as one gene from the synthetic lines was shown to be resistant to TPMK while the other was susceptible, and both *Sr30* and *Sr33* from Lorikeet are resistant to race TPMK (Jin, 2009; Rouse et al., 2011). If the Ug99 resistant gene in the synthetic lines is *Sr33*, no segregation would be observed. If the gene is not *Sr33*, we expected either a two dominant and one recessive gene segregation ratio of 61:3, or a three dominant gene ratio of 63:1, as Syn303 and Syn370 both have a dominant TPMK

resistant gene, and Lorikeet has one dominant TPMK resistant gene (*Sr33*) and one partially dominant TPMK resistant gene (*Sr30*). Both ratios could be possible, as it can be difficult to differentiate between a 61:3 ratio and a 63:1 ratio.

In the F<sub>2</sub> populations of Syn303/Lorikeet, 816 seedlings were screened, with 810 resistant seedlings and 6 susceptible seedlings observed, fitting our expected 63:1 segregation ratio of three dominant genes ( $\chi^2=3.63$ n.s.), but not a two dominant and one recessive gene ratio of 61:3 ( $\chi^2=28.62^{**}$ ).

Similar results were observed in the F<sub>2</sub> population of Syn370/Lorikeet with 624 plants screened, resulting in 623 resistant seedlings and 1 susceptible seedling observed. In this segregation, neither of our hypothesized gene ratios of 63:1 for three dominant genes ( $\chi^2=7.98^{**}$ ) or 61:3 for two dominant and one recessive gene ( $\chi^2=28.62^{**}$ ) fit this population. Segregation was observed in the F<sub>2</sub> generation in both populations, thus indicating that the TPMK/Ug99 resistance gene was most likely not homozygous and not the same in each parent. However, only one susceptible plant in Syn370/Lorikeet is not convincing evidence. This result meant *Sr33* was not in Syn303 or Syn370, assuming our source of Lorikeet contained *Sr33* (Park and Bariana, 2008; CIMMYT, 2012). These results did not support the original hypothesis of *Sr33*, commonly found in synthetic wheat, provided Ug99 resistance in the synthetic lines.

In both populations, we expected to see either a three dominant gene segregation or a two dominant and one recessive gene segregation, with two genes (*Sr33* and *Sr30*) coming from Lorikeet, and one gene coming from our synthetic lines. However, a three dominant gene segregation was only observed in the population of Syn303/Lorikeet, whereas a four dominant gene segregation was observed in both populations. This result

may indicate an epistatic interaction, chromosomal aberrations, or the presence of an additional gene within each population. We can only speculate on an additional gene in Lorikeet as we did not conduct any testing by crossing Lorikeet to a susceptible line, nor was an additional gene reported with Lorikeet. We did cross the synthetic lines to a susceptible line (Bill Brown), in which we determined that one TPMK resistant gene was present in Syn370. No seed was tested from the population of Syn303/Bill Brown, but we were able to determine that at least one TPMK resistant gene was present in Syn303 by crossing Syn303/Syn370. No segregation was observed when the progeny was tested with TPMK, indicating that at least one TPMK resistant gene was similar between the synthetic lines. However, this study could not determine whether Syn303 had one or two TPMK resistant genes. This result may help explain the four gene segregation in Syn303/Lorikeet, but not in the population of Syn370/Lorikeet.

To explain the difference between the Syn303/Lorikeet population and the Syn370/Lorikeet population, it is possible that some  $F_2$  plants were misclassified as resistant in the population of Syn370/Lorikeet, thereby producing an abundance of resistant lines and a lack of susceptible lines. It could also be possible that some  $F_1$  seed used to generate the Syn370/Lorikeet population were the result of a self pollination; hence the  $F_2$  seed included selfed and segregating seed, generating an abundance of resistant plants and a small quantity of susceptible plants in this population. It was necessary to use  $F_1$  seed from more than one plant in this population, as one plant alone could not supply enough seed for a population large enough to observe segregation. Using one hybridized seed and one self pollinated seed would account for the increased number of resistant plants in the Syn370/Lorikeet population.

If the abundance of resistant lines was not caused by misclassified lines or by using a combination of selfed and segregating seed, the previously identified TPMK susceptible gene within the synthetic lines could convey resistance in these populations, as both populations of Syn303/Lorikeet and Syn370/Lorikeet fit a four dominant gene segregation ratio, with Syn303/Lorikeet additionally fitting a three dominant and one recessive gene ratio. This result could mean that the expression of the TPMK susceptible gene identified in the Syn370/Bill Brown population was dependent on the genetic background that it is in.

The abundance of resistant lines in the population of Syn370/Lorikeet could also be explained by the presence of linked genes. We assumed that all genes assorted independently, but if *Sr33* or *Sr30* and the TPMK susceptible gene in Syn370 were linked in repulsion phase, we could see an abundance of resistant lines and a lack of susceptible lines in the progeny. An abundance of resistant lines was not observed in the population of Syn303/Lorikeet, which could indicate that the TPMK susceptible gene in Syn370 is not present in Syn303. TPMK was not used in testing with the population of Syn303/Bill Brown due to a lack of seed, so it was only postulated that the TPMK susceptible gene was present in both synthetic lines.

Based upon our data, we can say that the Ug99 resistance in Syn303 and Syn370 is not from *Sr33*. This can be inferred from the results of the Syn303/Syn370 cross indicating that the Ug99 resistant gene was identical in each population, and from observed segregation of seedlings in the populations of Syn303/Lorikeet and Syn370/Lorikeet indicating that the Ug99 resistant gene was not homozygous when crossed to Lorikeet, a cultivar containing *Sr33*. From this data we can say that the Ug99

resistance gene present in the synthetic line of Syn303 and Syn370 is an unidentified gene. The unidentified gene is a *Sr33*-like resistance gene because both genes result in an IT of 2,2+ when tested with TPMK and the Ug99 races of stem rust.

The identity of the other genes within the populations could not be confirmed, but an inference can be made on the possible identity of the TPMK susceptible gene present in Syn370. This gene can be narrowed down to dominant genes that are resistant to race QFCS but susceptible to TPMK, or a new gene. Previously identified genes that are resistant to race QFCS but susceptible to TPMK include *Sr7b*, *Sr9e*, *Sr11*, *Sr36*, or *SrTmp*. (Jin, 2009). *Sr36* can be ruled out, as that gene was transferred into common bread wheat from *T. timopheevii*. *Sr11* can likely be ruled out as well, as it is reported - that *Sr11* originated from the durum cultivar ‘Gaza’, though it is possible that Gaza could be in the background of the durum cultivars used in the creating of Syn303 and Syn370. *SrTmp* can also be ruled out as *SrTmp* is reported to originate from the hexaploid wheat cultivar ‘Turkey’. With those genes removed from consideration, the resistance gene could be *Sr7b*, *Sr9e*, or a new resistance gene. *Sr7b* is a possibility, as it is a common gene found in bread wheat (McIntosh et al., 1995). It is also possible that it is a new gene created in the cross to make synthetic wheat, but it is also possible that the gene is *Sr9e*. *Sr9e* is commonly found in durum wheat, and in a previous study *Sr9e* was reported from a cross between the durum cultivars ‘Laru’ and ‘Decoy 1’ with *A. tauschii* (Zulficar, 2008). The durum cultivars Laru and Decoy 1 were used to create the synthetic lines Syn303 and Syn370 in our study, so it is plausible that the cross that made our synthetic lines also resulted in *Sr9e*. No markers were available for *Sr9e*, and no crosses to

cultivars containing *Sr9e* were made, therefore we can only speculate on the true identity of this gene until further testing is completed.

This study aimed to provide insight into the genetic basis of stem rust resistance in three stem rust resistant lines (Goodstreak, Syn303, and Syn370). Results from the F<sub>2</sub> and F<sub>2:3</sub> generations suggest that two genes from Goodstreak are present in the population of GS274, with one being dominant (*Sr6*) and one being recessive, and three dominant genes and one recessive gene are present in the F<sub>2</sub> populations of GS303 and GS370. Through additional testing, we were able to determine that the resistance gene *Sr6* was present in all three populations (as expected), and that an unidentified *Sr33*-like resistance gene was present in the populations of GS303 and GS370. Though we were not able to verify the other genes present in the populations, we were able to hypothesize that *Sr9e* may be the TPMK susceptible gene in the population of GS370. The identification of resistance genes in the synthetic lines illustrates the value of screening germplasm for useful traits in addition to the ones they were selected for, as the synthetic lines used for this study were originally selected for drought tolerance as part of a previous study. By identifying two resistance genes, including a *Sr33*-like Ug99 resistance gene, and postulating an additional resistance gene, these sources of resistance can be used and effectively incorporated in future cultivars by plant breeders to provide additional resistance to North American races of stem rust, as well as the Ug99 family of stem rust.

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TABLES

**Table 1.** Plant Introduction number, UNL/CSU identifier, and pedigree of six synthetic hexaploid wheats from Onweller, (2011). Also listed are identifiers for *T. tauschii* donors at CIMMYT, KSU=Kansas State University, AUS=Australian Winter Cereals Collection, CPI=Commonwealth Plant Introduction Number, from Onweller, (2011)

<u>Synthetic Hexaploid</u>			<u>T. tauschii</u>			
PI#	UNL/CSU Identifier	Pedigree	CIMMYT wide cross #	KSU#	AUS#	CPI#
NSGC 9711	Syn166	Snipe/Yavaros79//Dackiye/Teal/3/Ae. Squarrosa(904)	904	2477	24154	110812
648646	Syn194	68112/Ward//Ae. Squarrosa (369)	369	2528	-	-
648733	Syn274	Garza/Boyer08//Ae. Squarrosa (241)	241	3282	24061	110719
648758	Syn303	Laru/ Ae. Squarrosa (333)	33	2482	24159	110817
648810	Syn356	Decoy 1/Ae. Squarrosa (256)	256	2400	24078	110737
648823	Syn370	Decoy 1/Ae. Squarrosa (322)	322	2471	24148	11086

**Table 2.** Reaction based on Stakman et al. (1962) of synthetic six synthetic hexaploid wheats to two North American and four Ug99 stem rust isolates. Infection types of 2 indicate resistance, with 3 and 4 indicating susceptibility, + indicating more sporulation, and – indicating less sporulation (Onweller, 2011)

CSU/UNL identifier	TPMK	TTTT	TTKSK	TRTT	TTKST	TTTSK
Syn166	2	2	2+	2	3+	4
Syn194	2-	4	4	2	n/a	n/a
Syn274	4	2+	4/2	4	4	4
Syn303	2	2	2+	2	2+	2+
Syn356	2	3	4	2	n/a	n/a
Syn370	2	2+3	2+	3	2+	2+

**Table 3.** Infection type based on Stakman et al. (1962) rating scale of parental lines to stem rust isolates QFCS and TPMK, with ;, 1, and 2 indicating resistance, and 4 indicating susceptibility.

CSU/UNL identifier	QFCS	TPMK
Syn274	4	4
Syn303	2	2
Syn370	2	2
Goodstreak	;	;
Bill Brown	4	4
Lorikeet	1	1
Thornbill	1	1

**Table 4.** Segregation of *Sr6* alleles at the Xcfd43 microsatellite marker locus in three F<sub>2</sub> populations of GS274, GS303, and GS370.

<b>Cross</b>	<b>N</b>	Observed			<b>Ratio</b>	Expected			$\chi^2$
		<b>Sr6Sr6</b>	<b>Sr6sr6</b>	<b>sr6sr6</b>		<b>Sr6Sr6</b>	<b>Sr6/sr6</b>	<b>sr6/sr6</b>	
GS274	198	55	99	44	1:2:1	49	99	49	1.22
GS303	100	19	55	26	1:2:1	25	50	25	1.98
GS370	102	15	52	35	1:2:1	26	51	26	7.88*

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

**Table 5.** Total number of plants screened (N), resistant (R), and susceptible (S) observed values, expected gene ratio for testing, the expected observation values and the resulting  $\chi^2$  value for F<sub>2</sub> individuals. Inoculated with stem rust race QFCS, unless noted in parenthesis.

<b>F<sub>2</sub> individuals</b>							
<b>Cross</b>	<b>Observed</b>				<b>Expected</b>		$\chi^2$
	<b>N</b>	<b>R</b>	<b>S</b>	<b>Ratio</b>	<b>R</b>	<b>S</b>	
GS274	214	153	61	15:1	201	13	180.89**
				13:3	174	40	13.37**
				3:1	161	53	1.4
GS303	103	99	4	15:1	97	6	0.98
				63:1	101	2	3.61
				61:3	98	5	0.15
				253:3	102	1	6.54*
GS370	106	99	7	15:1	99	7	0.02
				63:1	104	2	17.51**
				61:3	101	5	0.87
				253:3	105	1	27.00**
Syn303/Syn370	228	228	0	1:0	228	0	n/a
Syn303/Syn370 (TPMK)	96	96	0	1:0	96	0	n/a
Syn303/BB	77	70	7	15:1	72	5	1.06
Syn370/BB	116	110	6	15:1	109	7	0.23
Syn370/BB (TPMK)	121	95	26	3:1	91	30	0.80
Syn303/Lorikeet(TPMK)	816	810	6	63:1	803	13	3.63
				61:3	778	38	28.53**
				255:1	813	3	2.49
				253:3	806	10	1.34
Syn370/Lorikeet (TPMK)	624	623	1	63:1	614	10	7.98**
				61:3	595	29	28.62**
				255:1	622	2	0.85
				253:3	617	7	5.51*

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

**Table 6.** Observed values, expected gene ratio, the expected values, and the resulting  $\chi^2$  value for grouped ITs in F<sub>2</sub> progeny of the population GS274 when tested with stem rust race QFCS.

Cross	N	Observed			Ratio	Expected			$\chi^2$
		;1	2	4		;1	2	4	
GS274	214	117	36	61	9:3:4	120	40	54	1.57



**Table 7.** Resistant, segregating, and susceptible observed family values, expected gene ratio, the expected observation values and the resulting  $\chi^2$  value for the  $F_{2:3}$  families and  $F_2$  plant ratios based upon the  $F_{2:3}$  family ratios when inoculated with stem rust race QFCS.

F <sub>2:3</sub> families								
	Observed				Expected			
Cross	R	Seg	S	Ratio	R	Seg	S	χ <sup>2</sup>
GS274	115	57	37	9:3:4	118	39	52	12.60**
				9:4:3	118	52	39	0.61
F <sub>2</sub> plant ratios based upon the F <sub>2:3</sub> family ratios								
	Observed				Expected			
Cross	N	R	S	Ratio	R	S	χ <sup>2</sup>	
GS274	209	172	37	3:1	157	52	5.93*	
				15:1	196	13	46.79**	
				13:3	170	39	0.15	
GS303	99	97	2	15:1	93	6	3.02	
				63:1	97	2	0.13	
				61:3	94	5	0.32	
				253:3	98	1	0.62	
				255:1	99	0	6.76**	
GS370	102	101	1	15:1	96	6	4.83*	
				63:1	100	2	0.22	
				61:3	97	5	3.14	
				253:3	101	1	0.03	
				255:1	102	0	0.91	

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

**FIGURES**

**Figure 1.** Migration pattern observed with the *Sr6* linked marker Xcfd43 in the parental lines of Goodstreak, Syn303, Syn274, and Syn370 with the following patterns scored (L to R): Lane 1- 100 bp ladder; Lane 2- Goodstreak containing *Sr6* band at 215bp (indicated by arrow); Lane 3- Syn303 absent *Sr6*; Lane 4- Syn274 absent *Sr6*; Lane 5- Syn370 absent *Sr6*.

